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NUTRITION REVIEWS

May 1948

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WILLIAM J. DIECKMANN, M.D.
Chicago Lying-In Hospital
Chicago, Illinois

No one questions the fact that a healthy mother is more likely to give birth to a healthy infant. Good health is associated with a sufficient diet but there is a difference of opinion as to what constitutes such a diet. One has only to survey the food habits of various nationalities to realize that many nations have achieved great eminence and seem to be perfectly normal although their diets, as judged by nutritionally recommended food consumption of other countries, particularly the United States, seem to be lacking in sufficient amounts of various nutrients. Much of this divergence is undoubtedly due to unknown differences in food composition (R. S. Harris, *Nutrition Reviews* **6,** *33 (1948)*). Also it should be recognized that the body is able to compensate in various ways, most of which are not clearly understood, for minor deficiencies. If this were not so it is unlikely that the human race could have propagated to the degree that it has or reached the eminence that it has above all other species. But it is also possible that had the general nutrition of any country or race of people been definitely superior, that the health and accomplishments of the group would have been greatly improved.

Some internists question the value of the large amounts of milk and eggs (high lipoid content, especially cholesterol) which play so prominent a part in the diet instructions of our American nutritionists. Yet the experimental nutritionist continually demonstrates the superior nutritional qualities of these foods, the pediatrician tries to get the baby on a general diet as soon as possible, and the public health nutritionist advises adults to drink milk daily. In addition to what is known about a proper diet in pregnancy the problem is of such tremendous importance that further extension and study of the problem are highly desired.

There is no scientific proof that the pregnant animal or woman need gain more weight than the combined weight of fetus, placenta, amniotic fluid, term uterus and breast, and increase in blood volume. This total weight incidental to pregnancy is, at the most, 6.7 kg. If the patient is below her ideal weight, pregnancy is an excellent time in which she should gain this weight, but any excess weight is not only unnecessary but actually potentially dangerous. If the gain in weight of pregnant patients is kept to a maximum of 8 kg. above the ideal weight, the incidence of preeclampsia is decreased. The possibility of subsequent menstrual disturbances associated with obesity would also be decreased. There would probably be a lower incidence in later life of hypertension and the other complications that go with obesity.

It is well known that the pregnant woman needs increased amounts of calcium, phosphorus, nitrogen, and iron but the actual quantities are not well established and the recommended levels are based on limited studies. The National Research Council, after reviewing these various reports, has made certain recommendations for the diet of a normal pregnant patient. The recommended caloric intake may be too high for many of the patients during pregnancy and, if used, results in weight gains of 10, 15, or more kilograms.

The recommendations of the National Research Council, as well as the diet used at the Chicago Lying-In Hospital for normal pregnant, obese, and preeclamptic patients are given in the following table.

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DIET FROM THE VIEWPOINT OF THE OBSTETRICIAN

No one questions the fact that a healthy mother is more likely to give birth to a healthy infant. Good health is associated with a sufficient diet but there is a difference of opinion as to what constitutes such a diet. One has only to survey the food habits of various nationalities to realize that many nations have achieved great eminence and seem to be perfectly normal although their diets, as judged by nutritionally recommended food consumption of other countries, particularly the United States, seem to be lacking in sufficient amounts of various nutrients. Much of this divergence is undoubtedly due to unknown differences in food composition (R. S. Harris, *Nutrition Reviews* **6,** *33 (1948)*). Also it should be recognized that the body is able to compensate in various ways, most of which are not clearly understood, for minor deficiencies. If this were not so it is unlikely that the human race could have propagated to the degree that it has or reached the eminence that it has above all other species. But it is also possible that had the general nutrition of any country or race of people been definitely superior, that the health and accomplishments of the group would have been greatly improved.

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The recommendations of the National Research Council, as well as the diet used at the Chicago Lying-In Hospital for normal pregnant, obese, and preeclamptic patients are given in the following table.

Recommended Dietary Allowances	2500	85	1.5	15	6,000	1.8	2.5	18	100	400–800
Chicago Lying-In Diet										
Normal pregnancy	1808	85	1.44	16.3	20,327	1.6	2.9	17.8	139	400–800
1500-calory pregnancy	1495	84	1.46	15.7	19,811	1.5	3.1	12.6	144	400–800
Preeclamptic acid ash*	1823	85	0.76	15	9,196	1.2	1.4	17.4	124	400–800

* Preeclamptic diet: Sodium 0.472 g., Chlorine 0.572 g.

The Chicago diet is sufficient for the 60 kg. primipara who lives in a small apartment or home, and who is not athletically inclined. Obviously, the woman with a large family, or one who is working vigorously at home, in the factory, or elsewhere requires additional calories. A concentrate of cod liver oil is given daily to furnish approximately 400 to 800 I.U. of vitamin D.

If an adequate diet is only a fraction as important as some investigators have stated, the nutritionist is an important member of the prenatal team. Every pregnant woman should desirably be given careful diet instruction either by a doctor or by a nutritionist working in conjunction with the doctor. Periodic observations should be made of the patient's diet during pregnancy (not after delivery, when it is too late for corrective measures) to be certain that she is following instructions. It seems obvious that it is a waste of time to instruct patients if no attempt is made to determine just how effectively they follow the instructions.

Most obstetricians have no fixed opinion as to the degree of importance of diet in pregnancy, but wish to determine its value. Since 1944 a sufficient number of nutritionists have been available to instruct properly the patients in the Chicago Lying-In Hospital and, since 1946, to analyze the diet histories and make suggestions. For the past year a selected group of patients has been loaned a scale and taught how to use it. Periodically each patient weighs her food daily and records it at the same time. Twenty-four hour collections of urine for three day periods are made one to three times on most patients during pregnancy. These urines are analyzed for protein, nonprotein nitrogen, and creatinine. Thus a check can be made on the patient's nitrogen intake and her cooperativeness. A very small number of patients have kept analyses of intake and output for the calculation and determination of certain of the vitamins. Aliquots of the intake and the excreta are obtained by a trained nutritionist in the patient's home.

In general, most of these patients have had protein intakes less than 85 g. per day and comparatively few have been able to maintain consistently an increase in the protein intake. In a pilot study, dried milk and the several canned meat products available on the market were given without charge to patients, but were not found generally acceptable. We now have sixteen varieties of specially prepared meats (24 g. of protein per can) which are given to selected patients with the hope that the protein intake will be somewhere between 100 and 125 g. Sufficient quantities of these canned meats are available to supply 40 patients, each for a five month period. If it is found that enough patients will take the supplements regularly, a larger group will then be investigated with these supplements to determine how desirable a high protein intake is in pregnancy.

Studies on nutrition in pregnancy are being carried out in several other institutions and it is hoped that the results can be pooled and that definite conclusions based on a sizable amount of material can be reached which will indicate the degree of practical importance of diet in pregnancy.

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Chicago, Illinois

PRESENT KNOWLEDGE OF PTEROYLGLUTAMATES ("FOLIC ACID")

There are at least three chemically related compounds of nutritional importance which occur in natural products and which may be grouped together under the general term "pteroylglutamates." The deficiency syndrome which can now be related to a lack of pteroylglutamates has been recognized in the monkey since 1919 (P. L. Day, *Vitamins and Hormones* **2,** *71 (1944)*). The recognition of deficiencies of this vitamin by investigators who employed different experimental conditions has led to the application of a variety of names for the factor: vitamin M, factor U, factors R and S, vitamin Bc, norit eluate factor, folic acid, S. lactis R factor, and L. casei factor (see W. H. Peterson, *Ann. N. Y. Acad. Sci.* **48,** *257 (1946); Nutrition Reviews* **4,** *15 (1946)*). The term "folic acid" has been most widely employed and has sometimes been applied collectively to the group of pteroylglutamates, but at other times to designate a specific member.

Prior to the announcement of the synthesis of pteroylglutamic acid (PGA) (R. B. Angier *et al., Science* **102,** *227 (1945);* **103,** *667 (1946)*), the accumulated biologic evidence pointed to the existence of all three pteroylglutamates known at present (*Nutrition Reviews* **3,** *165 (1945)*), namely, vitamin Bc, Bc conjugate, and the fermentation factor.

The simplest pteroylglutamate proved to be composed of a pteridine grouping linked through a para-aminobenzoic acid residue to glutamic acid (*Nutrition Reviews* **4,** *199 (1946)*; Y. SubbaRow *et al., Ann. N. Y. Acad. Sci.* **48,** *255 (1946)*). The fermentation factor is a similar compound with two additional glutamic acid units in the side-chain; it may therefore be referred to as a triglutamate. Finally, vitamin Bc conjugate, prepared from yeast, was shown to have a similar structure but with a total of seven glutamic units in the side-chain (J. J. Pfiffner, D. G. Calkins, E. S. Bloom, and B. L. O'Dell, *J. Am. Chem. Soc.* **68,** *1392 (1946)*). These three compounds then may all be considered as derivatives of the pteridine-para-aminobenzoic acid compound to which the term pteroic acid has been given. If the carboxy group of this acid is linked with the amino group of one glutamic acid residue, the compound which results is pteroylglutamic acid. The arrangement of the additional glutamic acids in the side-chains of the conjugated forms has not been revealed, but the linkage is peptide in character (J. J. Pfiffner *et al., loc. cit.*).

Several tissues possess enzymes capable of converting the conjugated pteroylglutamates to pteroylglutamic acid (*Nutrition Reviews* **4,** *15 (1946)*). Pfiffner *et al.* (*loc. cit.*) identified the conjugase enzymes which split the heptaglutamate as carboxypeptidases. These enzymes are widely distributed in animal tissues and they may be concerned with the conversion of less available forms of pteroylglutamates to the simpler and more available PGA. The hypothesis that pteroylglutamic acid is the most active form of the pteroylglutamates, however, has not been proved.

Even before the identity of these glutamates was revealed, it was evident that in the nutrition of higher forms of life the conjugates and simpler substances were at least partially interchangeable (P. L. Day,

V. Mims, and J. R. Totter, *J. Biol. Chem.* **161,** *45 (1945)*) and it was suggested that the determination of "potential" folic acid might be of much greater nutritional significance than the determination of the preformed material (i.e., pteroylglutamic acid only) (*Nutrition Reviews* **5,** *246 (1947)*). It has now been demonstrated that pteroylglutamic acid, pteroyltriglutamic acid, and pteroylheptaglutamic acid are all active hematopoietically in man (*Ibid.* **5,** *168, 246 (1947)*). From the nutritional standpoint, therefore, it is of interest to collect data on the total pteroylglutamate content of foodstuffs and, since the relative activity of the pteroylglutamates may vary one from the other, to determine the amount of each form present in a given food material. To date the only methods available for such analyses are based on the judicious use of enzyme preparations combined with microbiologic assays.

Knowledge of the distribution of the pteroylglutamates in food materials is very incomplete. A summary provided by O. E. Olson, R. H. Burris, and C. A. Elvehjem (*J. Am. Dietet. Assn.* **23,** *200 (1947)*) indicates that fresh green leafy vegetables, cauliflower, kidney, and liver are rich in the vitamin; lean beef and veal, and prepared wheat cereals are intermediate in content; and root vegetables, tomatoes, light green leafy vegetables, bananas, pork, ham, lamb, cheese, milk, and canned foods are relatively low in "folic acid." These classifications must be considered as preliminary because of the inability to define the relative nutritional activities of the several forms of pteroylglutamates. The lability of this vitamin to the cooking processes has been compared with that of thiamine (*Nutrition Reviews* **4,** *286 (1946)*).

The pteroylglutamates are recognized as water soluble vitamins necessary for the monkey, chick (*Nutrition Reviews* **4,** *13 (1946)*), turkey (T. H. Jukes, E. L. R. Stokstad, and M. Belt, *J. Nutrition* **33,** *1 (1947)*), fox, mink (C. A. Elvehjem, "Symposium on Nutritional Anemias," *University of Cincinnati College of Medicine, Cincinnati, Ohio, Oct. 16–18 (1947)*), dog (*Nutrition Reviews* **5,** *205 (1947)*), the sulfa treated rat (*Ibid.* **2,** *103 (1944)*), guinea pig (D. W. Woolley and H. Sprince, *J. Biol. Chem.* **157,** *447 (1945)*), for pupation of mosquitos (*Nutrition Reviews* **3,** *173 (1945)*), and for growth of some microorganisms such as L. casei and S. lactis R. An experimental deficiency of pteroylglutamates has not been produced in man. Until this is done, it would perhaps be premature to state that man normally requires this vitamin. On the other hand, it would be surprising if it is eventually proved that he does not require it, especially in view of the undoubted activity of the vitamin in sprue, nutritional macrocytic anemia, pernicious anemia, and related macrocytic anemias. A deficiency of this factor in higher forms results in the development of an anemia and leukopenia of varying intensity. Thrombocytopenia may be encountered. The deficient animals also exhibit weight loss and, in some species, oral lesions and diarrhea. In the chick there is poor feathering and in the turkey a cervical paralysis occurs.

Sprue is a disease characterized by macrocytic anemia, moderate leukopenia, glossitis, diarrhea, weight loss, and evidences of defective gastrointestinal absorption. Administration of synthetic pteroylglutamic acid to patients with this disease has been followed by rapid and impressive remissions, both clinically and hematologically. The glossitis and diarrhea subside within a few days, a reticulocytosis occurs, and this is followed by regeneration of the erythrocytes and hemoglobin (*Nutrition Reviews* **4,** *11 (1946)*). In addition, it has been reported that the glucose tolerance curves of these patients change toward normal (W. J. Darby, E. Jones, and H. C. Johnson, *J. Am. Med. Assn.* **130,** *780 (1946)*) and that improved fat absorption occurs as indicated by gradual accumulation of carotene in the blood serum and alter-

ations toward normal of the vitamin A tolerance curve (W. J. Darby, M. M. Kaser, and E. Jones, *J. Nutrition* **33,** *243 (1947)*; Darby, Jones, H. F. Warden, and Kaser, *Ibid.* **34,** *645 (1947)*). In addition, there is slow but definite alteration toward normal in the gastrointestinal pattern of these patients as observed by x-ray (R. L. H. Beguerie and T. D. Spies, *Am. J. Roentgenol.* **56,** *337 (1946)*). It appears, therefore, that this vitamin is active in correcting the defects of both the hematopoietic and gastrointestinal systems in sprue.

L. S. P. Davidson, R. H. Girdwood, and E. M. Innes (*Lancet* **I,** *511 (1947)*) were also impressed with the excellent clinical responses of 6 cases of the sprue syndrome treated with PGA. They state: "Diarrhea was completely controlled within a few days of starting treatment and the patients all noted a rapid improvement in appetite and sense of well-being." These workers observed a variable hematopoietic effect in 5 of 7 cases but none of the hematologic values returned to normal on folic acid therapy. The red cell counts following therapy ranged from approximately 3.0 to 4.5 million. Several of these patients had been treated with liver prior to the period of folic acid therapy and had also failed to respond fully to that agent. J. F. Weir and M. W. Comfort (*J. Lab. Clin. Med.* **32,** *1231 (1947)*) report 7 cases of "nontropical sprue" which showed little evidence of permanent benefit from PGA. The initial red cell counts in this series ranged from 3.03 to 5.26 million per cubic millimeter and only one patient exhibited a megaloblastic marrow. Administration of PGA to this patient was followed by an initial hematologic and clinical remission but a relapse occurred after three months and the patient died. It seems likely, therefore, that the sprue syndrome consists of two or more disease entities: complete sprue which responds rapidly to PGA or liver extract and idiopathic steatorrhea in which the chronically altered intestinal function is refractory to folic acid or liver. Whether the latter is but an irreversible stage of the former remains to be determined.

In nutritional macrocytic anemia good responses have been obtained (G. A. Goldsmith, *J. Lab. Clin. Med.* **31,** *1186 (1946)*; T. A. Kemp, *Lancet* **II,** *350 (1947)*). It appears, therefore, that these two diseases, sprue and nutritional macrocytic anemia, which are usually associated with poor dietary habits, respond readily to small quantities (5 to 15 mg.) of pteroylglutamic acid either parenterally or orally. Although this finding suggests that these diseases may be due to a deficiency of this vitamin, they do not necessarily indicate that the deficiency is dietary in origin. The exact mechanism of development of a deficiency of pteroylglutamates in man is as yet undefined.

The good hematologic remissions brought about by administration of pteroylglutamic acid to patients with pernicious anemia (*Nutrition Reviews* **4,** *102 (1946)*) have been reported by a large group of investigators. These hematologic remissions resemble the remissions produced by administration of liver extracts. Much evidence, however, has now accumulated which demonstrates that the continuous administration of pteroylglutamic acid to patients with pernicious anemia does not protect these persons against the development of the disabling neurologic complications known as subacute combined sclerosis of the cord or combined system disease (B. E. Hall and C. H. Watkins, *J. Lab. Clin. Med.* **32,** *622 (1947)*; R. W. Heinle and A. D. Welch, *J. Am. Med. Assn.* **133,** *739 (1947)*; *Nutrition Reviews* **5,** *274 (1947)*). Furthermore, patients with pernicious anemia may exhibit initial hematologic remissions to PGA but later undergo a hematologic relapse despite continuous treatment with the factor (O. C. Hansen-Pruss, *Am. J. Med. Sci.* **214,** *465 (1947)*). These and other observations on patients with per-

nicious anemia justify two conclusions: (1) The mechanism of action of pteroylglutamic acid in patients with pernicious anemia is different from that of the active principle contained in antipernicious anemia liver extracts. (2) Pteroylglutamic acid cannot be considered a satisfactory or complete treatment of patients with pernicious anemia and should not replace potent liver extract as routine therapy until a satisfactory method for the protection of the nervous system is devised.

The exact mechanism of action of the pteroylglutamates in pernicious anemia is still unknown. It was initially suggested that the patient with pernicious anemia could not break down the conjugated forms of pteroylglutamic acid which are supplied by foodstuffs. Pteroylglutamic acid was assumed to be the hematopoietically active form of the vitamin and was unavailable to these patients (*Nutrition Reviews* **5,** *115* (*1947*)). Additional studies, however, have made this simple explanation untenable (*Ibid.* **5,** *208* (*1947*)). A recent investigation which may have a bearing on the defect which exists in pernicious anemia has been reported by M. E. Swendseid, O. D. Bird, R. A. Brown, and F. H. Bethell (*J. Lab. Clin. Med.* **32,** *23* (*1947*)). This is a study of the physiologic availability of various preparations of pteroylheptaglutamic acid (vitamin Bc conjugate) The amount of free pteroylglutamic acid excreted in the urine after the administration of the conjugate to human subjects was employed as a measure of the availability of the compound. In nature, vitamin Bc conjugate is often accompanied by a substance which inhibits the enzyme which converts the conjugate to pteroylglutamic acid. This inhibiting substance is known as conjugase inhibitor. Swendseid and her co-workers studied the excretion levels of healthy subjects following administration of the inhibitor-free conjugate alone and then during a period in which the subjects received the conjugate plus a source of this inhibitor. Simultaneous administration of the conjugase inhibitor and the conjugate resulted in lower excretion levels of PGA than occurred when the conjugate alone was fed. It would appear, therefore, that the conjugase inhibitor renders the conjugate less available to healthy subjects.

From the same laboratories (F. H. Bethell *et al.*, *J. Lab. Clin. Med.* **32,** *3* (*1947*)) have been reported studies on patients with pernicious anemia in relapse in which it appeared that some of the patients were unable to utilize the conjugated form of pteroylglutamic acid as measured by urinary excretion of free PGA. These same patients exhibited little or no hematopoietic response following treatment with the conjugate. Two patients, however, responded to the conjugate containing no inhibitor. The investigators postulated that in pernicious anemia this inability to utilize the heptaglutamate may depend, in part, upon the presence of the conjugase inhibitors present in the natural sources of conjugate. They further suggested that one of the actions of the liver principle might be the correction of the metabolic defect which results in improved utilization of the naturally occurring conjugated pteroylglutamates. A similar effect of liver extract principle upon the availability of conjugated pteroylglutamates was stated by R. M. Suárez *et al.* (*Ibid.* **31,** *1294* (*1946*)) to occur. An examination of the various data offered in support of this effect of the liver principle upon availability of conjugated pteroylglutamates reveals that all of the patients in whom this effect has been observed exhibited initial low urinary excretion levels of PGA. According to the usual convention in vitamin studies these patients would be considered to be deficient in this water soluble factor. The small amounts of the conjugate which were administered resulted in a gradual but definite increase in urinary excretion of free PGA in most cases. This increase was apparent prior to the administration of liver extract or of inhibitor-free conjugate. It appears

possible, therefore, that the phenomenon of tissue saturation has been given too little attention in these studies.

An interesting observation related to the studies of pteroylglutamates is that 5-methyl uracil (thymine), when administered in oral doses of several grams daily to patients with pernicious anemia, sprue, or nutritional macrocytic anemia, results in a hematologic remission which is definite but less in degree than remissions induced by administration of pteroylglutamic acid or liver extract (*Nutrition Reviews* **4,** *333 (1946);* W. B. Frommeyer and T. D. Spies, *Am. J. Med. Sci.* **213,** *135 (1947)*). Whether this observation indicates a basic relationship between PGA and thymine or merely that thymine is a vitamer of the pteroylglutamates is not clear.

In addition to the hematologic effect and the influence on gastrointestinal absorption in man, studies with chicks have revealed that the response of the oviduct to stilbestrol is dependent upon the presence of pteroylglutamates, a finding which indicates that these factors are involved in rapid tissue growth (*Nutrition Reviews* **3,** *308 (1945)*). Pteroylglutamic acid has also been identified with a factor concerned in lactation in the rat (*Ibid.* **5,** *311 (1947)*) and with the maintenance of resistance to endogenous infection in the monkey (S. Saslaw *et al., J. Exp. Med.* **84,** *263 (1946)*).

Antagonists for pteroylglutamic acid have been prepared. The most widely studied of these compounds is known as methylfolic acid, a substance which apparently has no hematopoietic activity for man in small doses but which increases the rate of appearance and severity of deficiency manifestations in the rat, mouse, chick, and pig (*Nutrition Reviews* **6,** *23 (1948)*). Another inhibitor known as pteroylaspartic acid acts as an antagonist in the chick but not in the rat (B. L. Hutchings *et al., J. Biol. Chem.* **170,** *323 (1947)*). The first of these compounds is described as a "displacing agent," the second was shown to be a competitive inhibitor. Finally, J. O. Lampen and M. J. Jones (*Ibid.* **164,** *485 (1946)*) have studied the inhibiting effect of sulfonamides on microorganisms and have concluded that the point of sulfonamide inhibition must be the synthesis of pteroylglutamic acid from para-aminobenzoic acid. Additional studies of the antagonists and antimetabolites will undoubtedly clarify many of the remaining problems in this complex field and may lead to valuable chemotherapeutic agents.

VITAMIN CONTENTS AND CHANGES OCCURRING IN FOOD PLANTS

As the knowledge of the chemical constituents of various plant foods increases, the ability to improve the native value of the food by genetic or cultural methods becomes possible. Numerous studies are in progress in these directions. Conditions of storage or processing of food are of equal importance, for a good food may deteriorate from the nutritional viewpoint through inadequate or unwise treatment before its consumption.

The nutritive value of bean sprouts is a subject of increasing interest in occidental countries. It might be expected that an item used for food so extensively in the Orient has survived because of good nutritional reasons, even though they might be unknown scientifically. A study of the vitamin content of soybeans as a function of germination·time was made by K. N. T. Wai, J. C. Bishop, P. B. Mack, and R. H. Cotton (*Plant Physiol.* **22,** *117 (1947)*). Analyses included carotene, thiamine, riboflavin, niacin, and ascorbic acid.

Sprouting was controlled under conditions known to be favorable to production of a good edible product. Earlier studies had

dealt less comprehensively with the problem, with little regard for edibility of the product. Also in the present study the hypocotyls and cotyledons were analyzed separately. Beans were soaked ten hours in a dilute solution of calcium hypochlorite at 21°C. to prevent mold growth before germinating at 28°C. Samples kept in cheesecloth over water were analyzed after twenty-four, forty-eight, fifty-four, and seventy-two hours of germination.

The thiamine content increased during the first twenty-four hours, then decreased sharply below the original value at fifty-four hours, increasing again at seventy-two hours. All other vitamins increased in contents (dry weight basis) to fifty-four hours and often up to seventy-two hours. Ascorbic acid increased the most, six- to sevenfold. Riboflavin and niacin increased up to 100 per cent. Carotene, originally low, increased slightly. The fifty-four hour period produced the superior product, from points of view of both edibility and appearance. Most of the vitamin changes had occurred in this period also.

A most significant finding was that the cotyledons contained much more of these vitamins than did the hypocotyls. This difference was threefold for thiamine and ascorbic acid, and fourfold for riboflavin, niacin, and carotene. The usual practice in oriental countries of discarding the cotyledons and eating only the hypocotyls would thus appear wasteful of these vitamins.

Vitamin patterns in the development of cucurbit fruits have been studied in detail by K. S. Wilson (*Am. J. Bot.* **34,** *469 (1947)*). Although this study was made from the point of view of a plant physiologist, the results are of direct interest to the purchaser of fresh vegetables for human consumption. The vitamins thiamine, riboflavin, and niacin were considered because of their function in enzyme systems participating in carbohydrate metabolism. Twelve races of two genera, including a commercial line of Hubbard squash, were employed. Ovaries were analyzed from a very small size to maturity of the fruit. Those larger than 5 mm. in diameter were sectioned into outer wall, inner wall, and placental region. Samples were dried at 70°C., ground, and analyzed microbiologically.

In all cases, niacin contents were higher (often tenfold) than those of riboflavin or thiamine. In most lines, relatively high vitamin concentrations were maintained until time of flowering, after which a decline occurred. As fruits reached maturity, differences were evident among lines; thiamine and sometimes niacin increased, while riboflavin remained low or decreased. Thus the early growth period, before flowering, is characterized by cell multiplication (and some enlargement) and maintenance of high vitamin levels; the latter period of growth is characterized by cell enlargement and reduction in vitamin concentration. Both large and small-fruited lines were studied and vitamin concentration was not a function of fruit size alone but rather of the stage of growth or developmental age of the fruit. At flowering time, types with different fruit (mature) sizes were similar in vitamin contents. After flowering, riboflavin and niacin decrease more sharply in the small-fruited types. In small-fruited woody lines, the rapid decline of niacin and riboflavin is concurrent with lignification of the outer wall. The most consistent pattern of vitamin behavior is found in the inner wall, which consists mostly of homogeneous parenchyma tissue.

Comparisons of diploid and tetraploid types indicated that at flowering and shortly thereafter, higher vitamin contents are generally found in the diploid races. In the placental region of tetraploids, thiamine did not increase at maturity, while a marked increase occurred in the diploid lines. Possibly this is related to the lower seed production of tetraploids.

From the nutritional viewpoint in relation to these vitamins, immature fruits of a large-

fruited type of squash are more desirable than more mature fruits of an equal size but from a small-fruited line. Cooking qualities and carbohydrate relationships would be expected to favor the former also.

Quantitative data are now available on the chemical changes occurring during storage in several varieties of the species commonly known as squash or pumpkin, through the work of T. G. Phillips (*Plant Physiol.* **21,** *533* (*1946*)). Three varieties were studied during storage periods of three to six months extending through three seasons. Initial composition of the edible portions of the varieties at harvest differed considerably, especially in the distribution of carbohydrates. Moisture contents differed widely. On the dry weight basis, total carbohydrate contents were equal and starch contents similar. However, total sugars differed as much as 100 per cent, as did sucrose content. Glucose varied fourfold and fructose twofold. The most striking changes occurred in sucrose content during storage. Variety Butternut, initially high in sucrose, increased twofold in two weeks and fivefold in three months. In other varieties fructose increased two- to threefold and glucose two- to fivefold. These increases in simple sugars occurred at the expense of starch. Variety Blue Hubbard attained high concentrations of glucose and fructose, as did Buttercup to a lesser degree, whereas Butternut decreased in its already low contents of these sugars but built up a sucrose concentration of 40 per cent (dry weight). Loss of carbohydrate through respiration was rapid, amounting to one-third and one-half after three and six months storage, respectively. Losses in protein nitrogen were slight. Thus, although losses of total carbohydrates are great during storage, this product increases considerably in sweetness due to the rapid hydrolysis of starch. Maltose was not found.

J. C. Walker and R. E. Foster (*Am. J. Bot.* **33,** *758* (*1946*)) have studied the inheritance of ascorbic acid content in cabbage. They report F_1 data from 23 crosses and F_2 data from 8 crosses between cabbage strains with high and low contents of ascorbic acid. In the F_1 hybrid progenies the mean content was intermediate between the mean values of corresponding parent progenies. In the F_2 progenies, the range was wider than for parent progenies, tested comparatively. Differences in standard deviations indicate that the parent plants differed in the genes governing ascorbic acid content. The authors conclude that ascorbic acid content is inherited as a quantitative character in cabbage and that the level of acid content attainable in a given line or individual is determined by a combination of genes, while the expression of the character may be influenced to some extent by environmental factors. Study of crosses indicates that by controlled crossing and selection the average ascorbic acid contents of the varieties of cabbage studied may be increased considerably while retaining resistance to yellows and desirable horticultural characteristics.

Certain Irish potatoes, when dehydrated, have a definite yellow color, due to naturally occurring carotenoid pigments. The Katahdin variety, when harvested in an immature state, as customary in the South, has a yellow color when the dried product contains 3 parts per million of carotenoids. This content may run as high as 7 p.p.m. in certain yellow-fleshed varieties. An intensive study of the identity of individual carotenoids found in Irish potatoes was made by B. C. Brunstetter and H. G. Wiseman (*Plant Physiol.* **22,** *421* (*1947*)). Great care was used in chromatographic separation of carotenoid pigments at low temperatures and in spectrophotometric measurements of the various fractions. A complex mixture of pigments was found, consisting of twelve zones from chromatography of the hypophase and at least nine zones from the hyperphase. Lutein, isolutein, a flavoxanthin, and probably auroxanthin were present. In the epiphasic fraction zeta-carotene was found to the extent of 0.1

p.p.m., dry weight. It is suggested that it is derived from beta-carotene by oxidation and is identical with aurochrome. More conclusive evidence is needed on this point. Its presence in many food items has been reported elsewhere. Beta-carotene was detected in concentrations of 0.3 p.p.m. and represents only about 10 per cent of the total carotenoids.

It would appear improbable from this work that carotenoids of yellow potatoes are of any significance nutritionally. The possibility remains that other varieties would yield different results in this regard.

INCREASED ASCORBIC ACID IN THE LIVER DURING PREGNANCY

E. L. Kennaway and N. M. Kennaway (*Cancer Research* **4**, *704* (*1944*)) studied the relation of pregnancy to the ascorbic acid content of the livers of seven strains of mice. In all, the livers of 53 pregnant and 56 nonpregnant females were studied. The seven strains represented were stock, Brown MRC, Buff MRC, C57, CBA, C3H, and dba. So far as possible, a pregnant and a nonpregnant mouse of the same breed and of similar age were killed on the same day as a safeguard against seasonal variation or changes in the composition of foodstuffs. The mean concentration of ascorbic acid in the liver was higher in the pregnant mice of each of the seven strains. The difference was not significant for the stock mice, was significant for the Brown and Buff MRC and dba strains ($P < 0.05$), and was highly significant in the C57, CBA, and C3H strains ($P < 0.01$). No distinction between strains of high (dba, C3H) and those of low mammary cancer incidence (C57, CBA) was seen in respect to the ascorbic acid content of the liver. In addition to the increase in ascorbic acid per gram of liver in the pregnant mice, there was an increase in the size of the livers at a rate proportional to the increase in the weight of the pregnant mice. The increased amount of ascorbic acid per gram and the increased size permitted about 50 per cent increase in the amount of ascorbic acid in the liver. Exact data on the relation of the ascorbic acid content of the liver to the stage of pregnancy were not presented.

E. L. Kennaway and M. M. Tipler (*Brit. J. Exp. Path.* **28**, *351* (*1947*)) made a similar investigation in two strains of rats, Wistar and black and white rats fed the ordinary mixed laboratory diet. Control and pregnant rats were killed on the same days. The beginning of pregnancy was roughly fixed by the observation of the vaginal plug. Some of the control rats were females which had not become pregnant during the mating period. The mean ascorbic acid contents of the livers of control and pregnant black and white rats were 248.5 and 289 micrograms per gram, respectively; the same values for Wistar rats were 221 and 262 micrograms per gram, respectively. Both of these comparisons represent significant differences. Unlike the mice, the percentage of body weight due to the liver was slightly increased during pregnancy. The increase in liver ascorbic acid tended to progress as pregnancy proceeded.

These data would seem to be of special significance since they were obtained in species which are able to maintain their own ascorbic acid supply. The question arises as to the importance to the pregnant animal of these appreciably increased amounts. A valuable fundamental problem would be to determine in rodents the effect of feeding amounts of glucoascorbic acid which were just sufficient to prevent these increases in liver ascorbic acid content during pregnancy upon the size, structure, and well-being of the offspring. So far as is known, the ascorbic acid metabolism of pregnant females of species which require ascorbic acid in their diets has never been

determined in terms of possible increases in liver storage nor is it known whether increased amounts of ascorbic acid are required to attain tissue saturation in pregnant females of the species requiring preformed ascorbic acid.

BIOTIN DEFICIENCY IN INFECTION WITH TRYPANOSOMES

A number of workers have described marked alterations in susceptibility to infectious agents as the result of various vitamin deficiencies. In earlier reviews an account has been given of some of these studies (*Nutrition Reviews* **3,** *345* (*1945*); **4,** *234* (*1946*)).

The report of F. E. Caldwell and P. György (*J. Infect. Dis.* **81,** *197* (*1947*)) is of special interest since a possible explanation for the phenomena observed is offered. All rats belonging to a specific experimental group and their controls were inoculated with the same number of trypanosomes per 100 g. of body weight. Counts were made, by appropriate methods, of the number of organisms in the blood of the animals and thus the rise and fall in the number of trypanosomes were measured. The degree of deficiency in the experimental rats was estimated by observing the extensiveness and severity of the classic signs of biotin deficiency. Autopsies were carried out at the end of the experimental periods and the rats were classified as having died as the result of bacterial infection, trypanosomal infection, or other (unexplained) causes.

In rats fed the stock Sherman (control) diet there were few deaths. Those that occurred in this group were due to Salmonella typhimurium infection which gained access to the colony at one time during the course of the experiments. There were no deaths from trypanosomal infection in these rats. In contrast, the markedly biotin deficient rats had more intense trypanosome infections with higher parasite counts than the controls. In many of these rats there was a progressive daily increase in the number of organisms in the blood until the time of death. In others, the rate of multiplication of the trypanosomes gradually decreased but was not completely inhibited until long after the controls. In several of these rats trypanolytic crises occurred. One of the animals successfully initiated a trypanolytic crisis but died with tremendous numbers of trypanosomes in the blood. The difference between the trypanosome infections in the slightly to moderately biotin deficient rats and in the control rats was less pronounced.

In a second experiment an attempt was made to determine the effect of an excess of biotin on the course of infection with Trypanosoma lewisi in biotin deficient rats. Beginning on the first day of the trypanosome infection and continuing each day thereafter, biotin deficient rats were each given 0.5 microgram of biotin subcutaneously. Daily injection of biotin did not prevent high numbers of trypanosomes from appearing in the blood of the biotin deficient rats. Only one of the rats showing signs of severe deficiency survived its infection.

In a third experiment rats were fed the biotin deficient diet but were given adequate biotin each day after weaning. In these rats the infection of T. lewisi was no different from that in the rats fed the Sherman diets.

To contrast the combined effect of a toxic factor, inanition, and a low protein intake on the course of the infection, a fourth experiment was carried out in which rats were given a linoleic acid diet. The rats fed this diet showed marked inanition and anemia and some died during the course of the experiment. Despite this, however, there was no increase in susceptibility to T. lewisi. The height of the infection in

the experimental rats was the same as in the controls.

A final study, of which only the preliminary results are reported, was one in which rats showing terminal signs of biotin deficiency were given "mature" or nonreproducing trypanosomes intravenously and were then given hyperimmune serum which had been so heated that complement would be destroyed. Twenty-four hours later none of the normal rats showed trypanosome infections whereas all of the biotin deficient rats were positive. Thus, rats suffering from terminal stages of biotin deficiency seemed to be unable to be protected from infections with T. lewisi by passive injections of hyperimmune serum in amounts that completely protected normal rats of the same weights.

These observations are interpreted in the light of the studies of W. H. Taliaferro (*Am. J. Hygiene* **16,** *32 (1932)*). Taliaferro demonstrated that ablastin, an antibody that inhibits the reproduction of trypanosomes, is a major factor in the development of immunity to T. lewisi in the rat. This antibody begins to make its appearance soon after the development of a patent infection. It reaches a passively transferable level when it inhibits the reproduction of the trypanosomes just before or at the time of the first lytic crisis. Ablastin may reach a high enough level to cause cessation of trypanosomal reproduction before any trypanolytic antibodies are produced in effective quantities. In such instances the trypanosomal population may remain stationary for a day or two, then suddenly drop due to the production of the initial trypanolysin which causes the death of most of the organisms. The surviving trypanosomes are thereafter resistant to the effect of the first lysin but are unable to multiply in the presence of the ablastin. Infections are finally terminated by the development of a second lysin.

Caldwell and György suggest that biotin deficiency results in a delayed and diminished production of ablastin. This conclusion is based on the observations that the trypanosomes continued to increase in number in the deficient animals after they had been already decreased in number in the control rats. The appearance of overwhelming numbers of trypanosomes without any inhibition of their reproduction, which led to the death of many of the markedly biotin deficient rats, would indicate that ablastin was poorly produced, if at all, in these animals. It was also observed that a delay occurred in the first trypanolytic number drop or "crisis" in all the biotin deficient rats when compared with the controls. The delayed crisis, which was especially pronounced in animals showing a severe biotin deficiency, is interpreted as indicating a decreased or delayed production of the initial trypanolysin by the deficient rats. The data indicate that excess biotin given at the beginning and continued throughout the infection did not affect the antibody producing mechanism in time to protect rats showing marked biotin deficiency. On the other hand, excess biotin given in this fashion to rats only slightly to moderately biotin deficient affected them sufficiently to cause termination as promptly as in the animals fed the Sherman diet. It would also seem that in severe biotin deficiency the lack of biotin may have caused a qualitative or quantitative deficiency in the complement of the deficient rats, thus making it impossible for the hyperimmune serum to offer any protective action. That the intensification and prolongation of the infections with T. lewisi in biotin deficient animals were caused specifically by the lack of biotin is indicated by the fact that such effects were not evident in rats given an identical diet and adequate biotin. Furthermore, inanition and a low protein diet *per se*, as produced in the rats fed the linoleic acid diet, did not lead to a pronounced lowering of the resistance of the albino rat to T. lewisi.

These observations do not indicate the

actual mechanism involved in the effect of biotin deficiency on antibody production. It is suggested that the lymphoid macrophage system may be affected by biotin deficiency in some way. W. H. Taliaferro, P. R. Cannon, and S. Goodloe (*Am. J. Hygiene* **14,** *1* (*1931*)), and others before them, showed that splenectomy reduces the ability of rats to cope with T. lewisi infections. Taliaferro and co-workers found that if rats were blockaded with India ink, as well as splenectomized, the production of ablastin was also diminished. Relapses of T. lewisi infection were much more likely to occur in splenectomized rats. Again, D. N. Naiman (*J. Parasitology* **30,** *209* (*1944*)) showed that rats, irradiated with x-rays so as to diminish the lymphoid tissue greatly, were not as able to produce antibodies to T. lewisi as normal rats.

COBALT DEFICIENCY AND VITAMIN LEVELS IN SHEEP BLOOD

Cows and sheep ingesting certain natural rations suffer from anemia and undergo a progressive emaciation, both of which are relieved by addition of minute amounts of cobalt to the ration (*Nutrition Reviews* **4,** *227* (*1946*)). For this reason, cobalt is listed among the trace mineral elements essential for animals. It has not, however, been possible to produce cobalt deficiency in nonruminant animals, even on diets as low or lower in cobalt than those which produce deficiency in ruminants. Little is known about the mechanism of action of cobalt in ruminant nutrition, except that it appears essential for proper hemoglobin formation.

S. N. Ray, W. C. Weir, A. L. Pope, and P. H. Phillips (*J. Nutrition* **34,** *595* (*1947*)) cite unpublished data which indicate that the effects of cobalt deficiency in ruminants can be partially alleviated by injections of thiamine and possibly of pyridoxine. This suggested that some of the effects of cobalt deficiency might result from lowered production of "B-vitamins" in the rumen, which might in turn result in lowered blood levels of certain of these vitamins. To check this possibility, the riboflavin, pantothenic acid, niacin, folic acid, and pyridoxine contents of the blood of sheep on cobalt deficient and sufficient rations were compared. The deficient animals used had, in most cases, been fed the low cobalt ration (containing <0.03 parts per million of cobalt) since birth, and all such animals had received this ration for from six months to one year at the time the comparisons were made. The normal animals were on the identical ration with added cobalt. In the deficient group, blood samples were taken only from those animals showing signs of incipient cobalt deficiency, as evidenced by a lowered growth rate, but at a time when their food intake was comparable to that of the controls. Blood samples from 6 to 13 animals were analyzed for each vitamin, all by microbiologic methods.

The results showed that the pyridoxine content of the blood was definitely lowered in the cobalt deficient animals (0.067 ± 0.010 vs. 0.118 ± 0.018 microgram per milliliter). This is interesting in view of the recognized role of pyridoxine as a coenzyme in many phases of protein metabolism, and because pyridoxine injections appeared helpful in ameliorating effects of cobalt deficiency. The niacin content of the deficient blood also appeared somewhat lower in the deficient animals (9.53 ± 2.99 vs. 12.14 ± 1.81 micrograms per milliliter), and there was a suggestion that the riboflavin content might also be lowered (0.235 ± 0.035 vs. 0.265 ± 0.028 micrograms per milliliter). In these latter instances, however, considerable overlapping in values from the two groups occurred. For the other vitamins, no differences were observed

in the two groups. In view of the cited efficacy of thiamine in ameliorating symptoms of cobalt deficiency, it is unfortunate that blood levels of thiamine were not determined. The study does, however, provide interesting leads for the further study and elucidation of the mechanism by which cobalt acts in the ruminant.

UTILIZATION OF PANTOTHENIC ACID AND OF BIOTIN DURING LACTATION

Although animal experiments have shown clearly the importance of both pantothenic acid and of biotin in nutrition, information concerning their role in man is meager. Since animal experiments have indicated the importance of pantothenic acid in reproduction and in the growth of young animals, M. N. Coryell and associates (*J. Lab. Clin. Med.* **32,** *1454* (*1947*)) decided to study the utilization of pantothenic acid from food by normal lactating women. This vitamin was measured in the food, milk, and urine during the first ten days postpartum and during five day periods at intervals during mature milk production. The report is part of an extensive study of the metabolism of women during the reproductive cycle which is being carried out by the Research Laboratory of the Children's Fund of Michigan.

The subjects were multiparas selected during the first trimester of pregnancy on the basis of physical and laboratory examinations and records of having successfully nourished their other children. During all periods of study the women received diets comparable qualitatively but varying in the quantity of all food except milk according to appetite. Aliquot portions of each food as eaten were weighed into a composite which was accumulated for each five day period of study. Both breasts were completely emptied by manual expression at the usual nursing intervals and samples taken for analysis from the complete twenty-four hour collection of milk. The entire excretion of urine during each twenty-four hour period also was obtained for analysis. Seven women were studied throughout the first ten days postpartum. Values were obtained for 3 other women on their seventh day following delivery. Five of the 7 women and 4 others were studied during seventeen five day periods while they were producing mature milk.

The average daily intakes of pantothenic acid ranged from 6.0 to 9.5 mg. During the first ten days postpartum daily secretion in milk rose with increasing volumes of milk produced. During the five days immediately following delivery the intakes of 6 women were 0.46 to 2.78 mg. in excess of secretion in milk and excretion in urine; during the next five days the milk and urine of 4 of the women contained averages of more pantothenic acid than their diets furnished. During the periods of mature milk production, 49 to 103 per cent of the intake was found in milk and urine. The urine alone contained 32 to 89 per cent of average daily intakes during the five day periods.

The influence of nutritional status and physiologic state upon the composition of breast milk was indicated by high concentrations in the milk of one woman who ingested multiple vitamin supplements throughout pregnancy in addition to an excellent diet and by the low values of another who developed a low grade infection near the end of the puerperium. The average daily urinary excretions of pantothenate for the various five day periods which Coryell and her associates observed are comparable to the average daily figures given by H. Spector, T. S. Hamilton, and H. H. Mitchell (*J. Biol. Chem.* **161,** *145* (*1945*)), and are somewhat higher than those of H. P. Sarett (*Ibid.* **159,** *321* (*1945*)), and others.

A similar study of biotin (M. N. Coryell

et al., J. Lab. Clin. Med. **32,** *1462 (1947)*) showed that the values for biotin in milk for the first four days following parturition were too low to be reliable. During the second five days both concentration and daily secretion of biotin in the milk varied widely among the 7 women, ranging from 0.24 microgram per day (0.015 microgram per 100 ml.) to 25.17 micrograms per day (2.56 micrograms per 100 ml.). During the periods of mature milk production averages of 2 to 14 per cent of the total biotin intakes (0.9 to 10.8 micrograms per day) were found in the milk secreted during the five day periods. From 15 to 70 per cent of the intakes (8 to 54 micrograms per day) were found in the urine.

It would seem from the data submitted that the maternal body can store pantothenic acid or that it is retained in the body for more than twenty-four hours during its metabolism. Apparently, physiologic levels of pantothenic acid are maintained in both the milk and urine of nursing mothers regardless of current intake if the women's nutritional status with respect to the vitamin is good. Since the nursing mothers receiving a diet providing ample amounts of pantothenic acid, excreted over 100 per cent of the net intake in urine, it seems unlikely that the feces play a significant role in excretion of pantothenic acid intake. With regard to biotin, considerable work indicates that fecal biotin is available to the body. Variations in fecal synthesis probably explain the wide range of response by individuals in a group.

ASCORBIC ACID AND ACCLIMATIZATION TO COLD

Until recently, little has been known from a physiologic standpoint concerning the real significance of acclimatization or adaptation except in the case of high altitude. The biochemical mechanisms and normal defense reactions which permit the organism to adapt to high or low temperatures is a field which has not been explored widely (*Nutrition Reviews* **3,** *187 (1945)*). It has been reported that there is a decrease in the ascorbic acid content of the tissue of guinea pigs when tissue is exposed, *in vitro*, to cold temperatures (D. Parvis, *Vitaminologia* **1,** *33 (1943)*) and others have found a decrease in ascorbic acid in the tissue of the same animal during the winter (E. Frommel and M. Loutfi, *Arch. Sci. Phys. Nat.* **26,** *16 (1944)*). Not only in animals, but also in plants the ascorbic acid content of the arctic plant is higher in northeastern Greenland than it is in the same species in southeastern Greenland (K. Rodahl, *Tr. Proc. Bot. Soc. Edinburgh* **34,** *205 (1944)*). The lack of beneficial effects of high ascorbic acid intake on the ability of man to work and to become acclimatized to heat also has been reported (*Nutrition Reviews* **3,** *5 (1945)*). Because of the rather fragmentary knowledge available, L. P. Dugal and M. Thérien (*Canad. J. Res. (Section E)* **25,** *111 (1947)*) undertook an investigation to determine whether ascorbic acid might be an important factor for resistance and adaptation to cold environment.

It was felt that the problem might be partly solved by studying the variations in the concentration of ascorbic acid in the tissues of white rats exposed gradually and of those exposed suddenly to low temperatures and to supplement this experiment by study of guinea pigs to observe the effect of administration of ascorbic acid, at cold temperatures, in animals unable to synthesize that vitamin, a property which is shared by man.

The work was presented in two separate parts, one dealt with white rats and the other with guinea pigs. The ascorbic acid content of the liver, kidneys, and testes of rats exposed and adapted to cold environ-

ments (4° to 0° C.) during different periods of time (one to six months) and of rats of the same age, fed the same diet but kept at room temperatures, were compared. More than 600 guinea pigs were placed into different groups and received daily, by oral administration, 0.5 to 75 mg. of ascorbic acid, respectively. Each group was then subdivided into two subgroups. One subgroup was exposed to cold and the other was kept at room temperature. Administration of ascorbic acid was always started at least three weeks before any group of guinea pigs was exposed to cold.

This study is reported in great detail with many charts and figures, and for details one is referred to the original article. In general, in the white rat an increase of as much as 80 per cent in the concentration of ascorbic acid occurred in the tissue exposed for long periods to cold temperatures. This increase was much smaller if the rats exposed to cold environments were given daily injections of ascorbic acid. Rats which died in the cold had a low content of ascorbic acid. The increase in the concentration of ascorbic acid was present regardless of the diet used but the increase was smaller with a diet rich in fat than with a diet rich in carbohydrates. Apparently, a diet rich in fat is better for survival and growth of rats in the cold environment than a diet which furnishes an equal number of calories but is rich in carbohydrates. At room temperature, the caloric intake of the rat was 70 calories per rat per day, while in the cold room it was 100 calories per rat per day. This was regardless of the type of diet used.

The extensive experiments on guinea pigs were performed to prove that for animals relying on exogenous sources of ascorbic acid this substance is necessary for resistance and adaptation to cold environments. The following results were obtained. Resistance and adaptation to cold temperatures, as measured by weight curves, depend on the amount of ascorbic acid received daily. Groups of guinea pigs were eliminated, or lost considerable weight, one by one, after the temperature was lowered. The first ones to be eliminated were the animals receiving 0.5 mg. of ascorbic acid daily and the last ones to be eliminated were those receiving 75 mg. daily. This resistance and adaptation to cold temperatures appear dependent on the concentration of ascorbic acid in the tissues, especially in the adrenal glands. Animals which were about to die at cold temperatures had much less ascorbic acid in the adrenal glands than those animals kept at room temperature. There was also some indication from this work that more ascorbic acid was retained in the adrenal glands of guinea pigs kept at cold temperatures during the first week of exposure to cold than in the adrenal glands of the control animals that were of the same age group and received the same amount of ascorbic acid daily but were kept at room temperature. This increase seems to be less after the animals have become fully acclimatized to cold. The same phenomenon had been observed in rats exposed to cold. When administration of ascorbic acid was discontinued during exposure to cold the survival in different groups of guinea pigs depended on the amount of ascorbic acid received previously. This was also true in animals kept at room temperature, but the survival was about twice as long at room temperature as at 0°C.

This study seems to indicate that ascorbic acid is necessary for resistance and adaptation to cold environments for animals, such as guinea pigs, which are unable to synthesize this vitamin. It is also of some interest that the ascorbic acid content of the urine of man is less when the environment is colder (*Nat. Res. Council of Canada, Report C-6256 (1946)*).

This extensive research opens up a tremendous field of investigation concerning the biochemical and physiologic mechanisms involved in the adaptation of animals and perhaps of man to cold and high temperatures.

LIPOTROPIC ACTIVITY OF CAFFEINE, THEOBROMINE, AND THEOPHYLLINE

Despite the wide use of the methyl purines in medical practice, little is known of their metabolic fate. Recent investigations indicate, however, that these substances are partially oxidized and partially demethylated, and may thus be excreted in part as methyl uric acids (V. C. Myers and R. F. Hanzal, *J. Biol. Chem.* **162,** *309 (1946)*). Whether these substances can contribute to the metabolic pool of essential methyl groups is unknown. A. W. Moyer and V. du Vigneaud (*Ibid.* **143,** *373 (1942)*), however, found that caffeine did not permit growth of rats on a methionine free and choline free diet containing homocystine, and presumably could not, therefore, methylate this substance. On the other hand, C. A. Mills and E. Cottingham (*Arch. Biochem.* **4,** *171 (1944)*) reported that caffeine did protect rats from the hemorrhagic nephritis of choline deficiency, albeit somewhat less effectively than methionine.

L. A. Heppel, V. T. Porterfield, and E. G. Peake (*Arch. Biochem.* **15,** *439 (1947)*) report results of an experiment designed to determine whether these compounds possess lipotropic activity, as do choline and methionine. Weanling rats were fed a low protein (6 per cent purified casein), high fat (38 per cent hydrogenated cottonseed oil) ration of the synthetic type, with salts and vitamins, but deficient in choline. Control groups received this diet for twenty-one to twenty-seven days; experimental groups received 0.3 to 0.6 per cent caffeine, theophylline, or theobromine in the ration for the same time. During this time, animals of the control groups just maintained their original weights; those fed methyl purines did not maintain their weight, but lost from 10 to 20 g. (initial weights about 46 g.) during the experimental period. Theobromine and caffeine produced no decrease in food intake, and no symptoms of intoxication except weight loss; the rats fed theophylline showed markedly decreased food intake and a correspondingly larger decrease in weight. At the end of the experimental period, the livers were weighed and analyzed for total fat (chloroform soluble fraction of the hot alcohol-ether extract). Livers from the control rats weighed 3.9 to 4.2 g., and contained from 28 to 32 per cent fat; livers of animals fed the methyl purines weighed from 2.1 to 2.7 g. (theophylline group, 1.15 g.), and contained 9.5 to 17.3 per cent fat. The marked lipotropic action of the methyl purines is apparent from these figures.

Unfortunately, a direct comparison of the degree of lipotropic action of methyl purines with that of methionine or of choline was not made. In a separate experiment, however, 1 per cent of added methionine, equivalent in methyl content to 0.47 per cent caffeine, reduced the fat content of the livers to 10.5 per cent, roughly equivalent to the action of 0.3 to 0.4 per cent of caffeine. Smaller amounts of methionine were not tried.

The mechanism of the lipotropic action of these compounds is not known. The authors state that separate experiments have confirmed the results of Moyer and du Vigneaud (*loc. cit.*) that the methyl purines do not permit methylation of homocystine by choline deficient rats. Their action in preventing fatty livers and hemorrhagic kidneys may perhaps be likened to that of dimethylaminoethanol, since this compound is similarly effective in these conditions, but is relatively inefficient in substituting for choline in methyl free diets (V. du Vigneaud *et al.*, *J. Biol. Chem.* **164,** *603 (1946)*). It may be pointed out that although the apparent ability of these methyl purines to furnish methyl groups to the body is of considerable theoretical interest, it is scarcely of practical importance from the standpoint of nutrition.

METABOLIC EFFECTS OF IMMOBILIZATION IN MAN

Metabolic alterations following infection and trauma and in convalescence were the object of intensive investigations during World War II. Many of the more recent contributions to this study have been reviewed (*Nutrition Reviews* **3,** *40, 86, 253 (1945)*; **4,** *6 (1946)*; **5,** *46 (1947)*). Following fracture, operations, and burns, a "catabolic period" of variable duration usually appears and is followed by a subsequent "anabolic period." During the period of catabolism the chief abnormality is a persistently negative nitrogen balance and consequent loss of body tissue. During the anabolic period a reversal of the metabolic alterations occurs, particularly evident being a period of positive nitrogen balance. The retained nitrogen is assumed to be making up for the body deficit incurred during the catabolic period. On the basis of these and other observations it was postulated that trauma stimulated the elaboration of the adrenal cortical "S" hormone which presumably increased the amount of available glucose by breakdown of protein and deaminization of amino acids. During the anabolic period, the contrary changes (i.e., deposition of protein) were thought to be brought about by elaboration of the anabolic adrenal cortical "N" hormone.

The metabolic results of these investigations have been repeatedly confirmed, but their significance and interpretation can only be surmised until control of all the possible variables has been accomplished. Immobilization, either simple bed rest or enforced by the application of plaster casts, is an important variable and would appear to be susceptible to further study. Furthermore, such an investigation would help clarify recent criticisms of prolonged rest and inactivity (*J. Am. Med. Assn.* **124,** *1075 (1944)*). In a carefully planned and executed study, J. E. Deitrick, G. D. Whedon, and E. Shorr (*Am. J. Med.* **4,** *3 (1948)*) have studied the effects of immobilization upon various metabolic and physiologic functions of normal men.

Four healthy young men were studied during a period of six to seven weeks of bed rest which was controlled by immobilization from the midabdomen down in a bi-valved plaster cast. Control periods of six to eight weeks before, and four to six weeks after immobilization were included. The diets were constant throughout the study and furnished daily fom 2500 to 2800 calories, 85 to 90 g. of protein, 110 to 114 g. of fat, 292 to 352 g. of carbohydrate, 852 to 920 mg. of calcium, 1.50 to 1.64 g. of phosphorus, and 3.15 to 3.76 g. of potassium. The quantities of other nutrients provided are not given (except for ascorbic acid which averaged 144 mg. daily). Extensive biochemical, physiologic, and balance studies were carried out, generally by well accepted methods.

Nitrogen balance was slightly positive (about 1 to 2 g. daily) during the control period, while in the period of immobilization it approached or reached equilibrium in 2 subjects and was actually 1 to 2 g. per day negative for about three weeks in 2 subjects. During the afterperiod, positive nitrogen balance, more marked than in the foreperiod (2 to 3 g. daily), was observed. The average total nitrogen loss during immobilization was 57.3 g., an amount about one-quarter to one-fifth as great as had been found by others following fractures (J. E. Howard *et al., Bull. Johns Hopkins Hosp.* **75,** *156 (1944)*).

Calcium and phosphorus balances likewise were slightly positive during the fore-(control) period. During the period of immobilization both urinary and fecal calcium and phosphorus excretion increased so that negative balance was evident in each subject, at least for a short time. In 2 subjects the extent of the negative calcium balance was marked. The control level of

calcium balance was not reached until about three weeks after the resumption of activity. The total calcium lost, including the first three recovery weeks, varied from almost 9 to 24 g. and averaged 14.1 g., a value roughly one-half that reported by Howard *et al.* (*loc. cit.*). Symptoms or urinary findings suggestive of renal calculus formation were not observed despite the combination of a high calcium excretion and lack of such protective changes as increase in urinary volume, decrease in pH (it actually became more alkaline), and increased excretion of citric acid—a combination which appears to be optimal for stone formation (E. Shorr *et al.*, *Science* **96,** *587* (*1942*)). Phosphorus retention also occurred after activity was resumed. Theoretical phosphorus losses calculated from the calcium and nitrogen losses agreed fairly well from week to week although the total theoretical and actual losses deviated from each other by 5.1 to 23.4 per cent.

There was a close correlation between the extent of the urinary sulfur and nitrogen excretion with an average ratio of urinary sulfur to urinary nitrogen of about 1:15.8, suggesting, the authors point out, a sulfur-rich source of nitrogen during the period of immobilization which was presumably muscle. It should be noted, however, that the ratio was the same during the foreperiod when there was presumably no specific wasting of muscle tissues. Potassium balance and excretion (the changes were entirely in the urine) only roughly followed those of nitrogen and sulfur. As further evidence of diminished muscle mass, the authors found a diminished retention when the subjects were fed creatinine, and definite evidence of muscular weakness. The urinary excretion of 17-ketosteroids and adrenal corticoids (glycogen deposition in mice) did not show consistent changes. Thus, the negative nitrogen, sulfur, and potassium balances and the increased creatine tolerance strongly suggested that muscular atrophy was occurring in these individuals during the period of immobilization. Actual measurements showing a decrease in the girth of the extremities substantiated the loss of muscle tissue and further revealed that it was more marked, as one would expect, in the legs than arms, the area to which the cast was applied. During the period of immobilization the basal metabolic rate fell an average of 6.9 per cent. Body weight was almost constant.

By use of the tilt-table technic impairment of the mechanisms for maintaining the circulation when the exact posture was assumed was demonstrated. This appeared to be due chiefly to a fault in the lower extremities. The fainting in one individual, after suddenly assuming the erect posture, for example, could be abrogated by the previous application of elastic bandages.

Dependent purpuric hemorrhages (presumably petechiae) appeared in all subjects near the end of the period of immobilization. This was thought to be due to the circulatory disturbances rather than to a change in the humoral factors responsible for hemostasis. Blood clotting times, prothrombin times, and platelet counts were normal throughout.

The absence of abnormally accelerated blood clotting factors and the fact that phlebothrombosis did not occur during immobilization are mentioned by the authors as evidence against immobilization *per se* causing the formation of intravascular clots. However, it should be pointed out, that these tests are but crude measures of blood coagulability, particularly when increased, and that failure of phlebothrombosis to occur in 4 individuals does not appear unusually significant.

The occurrence of certain specific metabolic changes associated with enforced rest alone in healthy subjects cannot be doubted. However, as the authors point out, the effects are not great (for example, nitrogen and calcium losses were from one-quarter to one-half those seen following fractures) and are reversible and, further, few hospital patients are immobilized to

this degree for this length of time. When this extent of immobilization does occur, however, the possible formation of renal calculi, the impaired response to the assumption of erect posture, and the loss of muscle tissue and strength may be hazards, particularly when added to the abnormalities associated with the specific illness concerned

AMINO ACID REQUIREMENTS OF THE CHICK

Chicks will grow normally if an otherwise adequate diet supplies the following levels of essential amino acids (values represent percentage of the diet): arginine, 1.2; methionine, in addition to an adequate intake of cystine, 0.5; methionine, in the absence of dietary cystine, 0.9; lysine, 0.9; tryptophan, 0.25; leucine, not more than 1.4; phenylalanine, in addition to an adequate supply of tyrosine, 0.9; phenylalanine, in the absence of dietary tyrosine, 1.6; threonine, 0.6; valine, 0.8; isoleucine, 0.6; and, histidine, not more than 0.3 (H. J. Almquist, *J. Nutrition* **34,** *543 (1947)*). These requirements were established as the result of a comprehensive analysis and evaluation of numerous investigations by H. J. Almquist and by others interested in poultry nutrition. Certain of these studies have been discussed in previous reviews (*Nutrition Reviews* **3,** *100 (1945)*; **5,** *57 (1947)*).

Reproducible growth data are readily obtained by adherence to the following specifications: uniformity in the breed or strain of the experimental chicks; inclusion of a ten to fourteen day pretest rearing period on an excellent practical diet prior to a ten day test period; and, use of an otherwise adequate diet fed ad libitum and containing at least 20 per cent of dietary protein or its amino acid equivalent. Under these conditions the rate of gain or the percentage daily change in body weight, bears a linear relation to the percentage concentration of the limiting amino acid in the diet. This relationship holds for all levels from zero to the optimal concentration. Data plotted in this manner indicate that maintenance, or zero rate of growth, occurs if a diet supplies approximately 30 per cent of the optimal levels of amino acids. Diets containing one half of the optimal concentrations permit a rate of growth which is roughly two sevenths of normal.

The absence of an essential amino acid from the diet results in loss of weight. It is significant that the rate of loss is about 3 per cent regardless of which amino acid is lacking and that this value agrees well with the rate of loss on diets devoid of protein and amino acids. Failure of a diet to furnish a single necessary constituent appears to prevent protein synthesis as effectively as a multiple amino acid deficiency. Under the standard conditions listed above, a rate of loss which is less than 3 per cent daily actually represents a state of improved protein synthesis or, at least, a greater sparing of tissue protein. For this reason Almquist suggests that growth rates that are affected by protein synthesis should be compared with the 3 per cent rate of loss as a base line rather than with zero growth as the standard. This is reasonable because net gain in weight must depend upon the balance between anabolism and catabolism of protein.

It is noteworthy that there is close agreement between the maximal growth effects of individual amino acids whether supplied as free acids or as protein. This fact and the linear nature of the growth curves for both types of diets indicate that the requirement of any one indispensable amino acid for a specified rate of growth bears a fixed relationship to the remaining essential amino acids. This is compatible with the hypothesis that the synthesis of protein in the growing animal always requires the

same proportions of building materials. The observations further imply that the proportions of amino acids reaching the tissues where synthesis into protein occurs are dependent upon the proportions in the diet. It should be noted that the above concept does not recognize a possible injurious effect of imbalance of amino acids in the case of mixtures which provide an excess of one and optimal quantities, or more, of all.

Even though essential amino acids have the same maximal growth effects when supplied as amino acid mixtures, hydrolysates, or protein, the rate of gain of chicks on the diets containing whole protein is generally greater. Almquist believes that this superiority of unhydrolyzed protein is explained in large part by a rapid inhibition of appetite in the case of chicks fed amino acid mixtures or hydrolysates. Chicks on a diet containing fish protein grew at the same rate when restricted to two two-hour feeding periods daily as when food was always available. The rate of growth was decreased 40 per cent on the periodic feeding regimen if the fish protein was hydrolyzed before incorporation into the diet. The rate was diminished only 12 per cent on the same diet without a time restriction. The food consumption of each group supplied whole protein and of the unrestricted group supplied hydrolyzed protein, was practically the same. The group fed hydrolyzed protein during two two-hour feeding periods ate appreciably less food, presumably because the more rapid absorption of amino acids induced a faster physiologic curtailment of appetite. The same inhibition of appetite would occur in each group fed the hydrolyzed protein but would be partially offset in the unrestricted group because it was possible for those chicks to resume eating at any time.

Other factors may also contribute to the more rapid gains observed on diets containing whole protein. The slower absorption of amino acids liberated by digestive processes in the alimentary canal may result in less wasteful catabolism and in smaller urinary and fecal losses. It is possible that the absorption of simple peptides may facilitate protein synthesis but Almquist does not believe that the accumulated data necessarily support the conclusion that a special growth factor for chicks is present in animal proteins and lacking in the same proteins after complete hydrolysis (*Nutrition Reviews* **4,** *273 (1946)*).

Knowledge of the quantitative requirements of amino acids in growing animals is a prerequisite for further advances in protein nutrition. Chicks and young rats are particularly useful in such studies. In both species, the list of indispensable amino acids is the same, and methionine and phenylalanine are partially spared by cystine and tyrosine, respectively. Glycine is synthesized but the rate of synthesis is limited in the rat as well as in the chick. This may also be true of glutamic acid synthesis in the chick (*Nutrition Reviews* **3,** *100 (1945)*). The extension of the findings in the chick to the corresponding requirements of the adult fowl for maintenance and for production of hatchable eggs will be highly instructive and economically important.

FATTY ACID DEFICIENCY IN MATURE RATS

The syndrome produced by feeding young rats on rigorously fat free diets has been produced frequently, and has led to recognition of linoleic and arachidonic acids as "essential fatty acids" (*Nutrition Reviews* **5,** *258 (1947)*). In no case, however, has the deficiency syndrome been produced in mature animals. This has now been accomplished by V. H. Barki, H. Nath, E. B. Hart, and C. A. Elvehjem (*Proc. Soc. Exp. Biol. Med.* **66,** *474 (1947)*).

Mature male Sprague-Dawley rats were

maintained on a fat free, synthetic-type ration (fat free casein 30, sucrose 66, salts 4, synthetic B-vitamins, and 1 drop of haliver oil weekly). Separate groups received different levels of "B-vitamins" ranging from adequate to very high. One group received corn oil, a source of unsaturated fatty acids, in addition. For two months the food intake was restricted to 6 g. of food per rat per day, and was then reduced to 5 g. per day. As a result of the severely restricted caloric intake, all animals lost weight; on the ninety-first day one animal from each of the fat free groups died at weights of 94, 93, and 95 g., compared with initial weights of about 225 g. At this time, all animals were allowed their respective diets ad libitum. The only symptom other than severe emaciation observed in the animals at this time was a slight "rustiness" of the fur about the neck and shoulders.

After sixteen days on ad libitum feeding, various skin symptoms were observed in the animals receiving the fat free rations. Scaly hind paws were most uniformly present; other symptoms occurring in some animals included necrosis and loss of the tip of the tail, scaly tail, annular tail, loss of skin near the base of the tail and around the mouth, loss of hair around the mouth, eyes, over the forepaws, at the base of the tail, and around the scrotum. Thinness of the hair coat over the back, chest, and abdomen was apparent; some edema of the fore and hind paws was apparent and prolapsed penis. A group of animals in which 10 per cent of the casein of the ration was replaced with 10 per cent of dried brewers' yeast developed the same symptoms slightly later than the two groups on the synthetic fat free rations. By contrast, the group receiving corn oil showed practically none of these symptoms at any time. The control was not ironclad, however, since the animals receiving corn oil, in contrast to rats on the fat free rations, were also given certain "B-vitamins" not ordinarily required in the diet of rats fed synthetic rations (biotin, folic acid, inositol, niacin, and para-aminobenzoic acid). A curative experiment was therefore begun.

The animals which developed symptoms on the fat free ration were divided into three groups. One group was maintained on the fat free control ration, one was given corn oil, and one was given the extra five vitamins listed above. In two weeks those animals receiving corn oil showed signs of recovery and were completely cured in five to six weeks. Those animals not receiving corn oil still showed symptoms of the deficiency at seven weeks, when the experiment was terminated. Separate experiments showed that ethyl linoleate was the equivalent of corn oil, i.e. that the effect of corn oil was duplicated by essential fatty acids, and was hence presumably due to its content of these acids.

For development of the deficiency syndrome, preliminary caloric restriction with its accompanying weight losses, followed by ad libitum feeding, were essential. Animals maintained on the fat free rations and fed ad libitum throughout the whole experiment never developed any of the symptoms of fatty acid deficiency. Furthermore, the starved animals which developed the deficiency following ad libitum feeding were gradually cured spontaneously when maintained on the same fat free ration for periods up to ten weeks. This suggests the possibility of a slow intestinal synthesis of the essential fatty acids.

Although the deficiency has thus been produced in mature animals, it has been done only following starvation, when the mature animal, like the young rat, must meet the heightened requirements of both maintenance and growth. Examination of the growth data show that, in addition to preventing or curing symptoms of essential fatty acid deficiency, corn oil or ethyl linoleate permitted faster achievement of normal weight by the starved animals.

The authors suggest that the starvation method employed in this investigation is suitable for wider application where it is desired to produce increased nutritive requirements by imposing a "strain" on normal bodily requirements.

EFFECT OF MALNUTRITION ON GROWTH AND PHYSICAL STATUS OF CHILDREN

The influence of inadequate food intake upon the growth and physical condition of children is notoriously difficult to evaluate. Many factors other than nutrition affect growth and physical condition; for example, sunlight and exercise, as well as disease, communicable, and other. A. W. Mann, S. Dreizen, and T. D. Spies (*J. Pediatrics* **31,** *161* (*1947*)) attempted to reduce the number and significance of these variables by comparing over a two year period two groups of 47 children each presumably comparable in heredity and geographic environment but differing widely in socio-economic status and in food habits.

The children in one group who came from rural areas were from parents who were not only economically impoverished but who, themselves, showed signs and symptoms of nutritional deficiency as observed by T. D. Spies and colleagues at the Nutrition Clinic. Even the domestic pets of these families showed classic signs of deficiency disease. The other group of children, used as controls, came from urban areas, and were free from nutritional deficiency. The family incomes were high enough to permit adequate diets.

The diets of the two groups of children were compared for content of protein, riboflavin, thiamine, niacin, calcium, phosphorus, iron, ascorbic acid, and vitamin A. The food eaten by the two sexes in both groups was classed as adequate, borderline adequacy, or deficient, the latter two being defined as close to and below the National Research Council recommendations, respectively. In general, there were occasional instances of borderline and only one instance of a deficiency of one of the nutrients in the control group, while gross deficiencies were present in a large number of the deficient group. The authors do not describe their methods for assessing food intake nor is any comment made on the use of the recommended daily allowances of the National Research Council as standards of dietary adequacy.

Comparison of the incidence of the signs and symptoms suggestive of nutritional deficiency between the two groups during the two year period of study revealed a striking parallelism with the dietary intakes. Thus, the children whose diets were inferior had a high incidence of these suggestive signs and symptoms, while the control group was essentially free of them. Both groups had the same incidence of allergic disturbances, but the control group had almost twice as many acute infectious diseases as did the malnourished children, because of their urban environment the authors suggest.

Further study of the children of the two groups was made using the "Wetzel Grid." Interpretation of these observations would seem to indicate that the rate of development of the two groups was not significantly different, although more of the children in the malnourished group were classed as being in fair, borderline, or poor physical status. However, the basal daily heat production of the two groups did differ, the malnourished children having a definitely and progressively lower heat production for their age than the control children. The authors believe that "dietary inadequacies" are responsible for failure to maintain normal basal heat production for the age, although

maintained on a fat free, synthetic-type ration (fat free casein 30, sucrose 66, salts 4, synthetic B-vitamins, and 1 drop of haliver oil weekly). Separate groups received different levels of "B-vitamins" ranging from adequate to very high. One group received corn oil, a source of unsaturated fatty acids, in addition. For two months the food intake was restricted to 6 g. of food per rat per day, and was then reduced to 5 g. per day. As a result of the severely restricted caloric intake, all animals lost weight; on the ninety-first day one animal from each of the fat free groups died at weights of 94, 93, and 95 g., compared with initial weights of about 225 g. At this time, all animals were allowed their respective diets ad libitum. The only symptom other than severe emaciation observed in the animals at this time was a slight "rustiness" of the fur about the neck and shoulders.

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the reason why simple caloric reduction might not account for this phenomenon is not made clear. It does not appear to be possible, from the data presented, to relate more clearly the physical development and nutritional status of these children than to say that generally they paralleled each other. It could not be decided whether good or poor food intake was responsible for the respectively good or poor physical status nor "the extent to which the growth and physical condition of these children was influenced by nutritive failure," the authors' professed objective.

NUTRITIVE VALUE OF ALBUMIN

Considerable work has been published concerning the usefulness of human serum albumin administered intravenously in shock and in some of the diseases in man associated with hypoproteinemia (C. A. Janeway *et al.*, *J. Clin. Invest.* **23,** *465* (*1944*); A. Cournand *et al.*, *Ibid.* **23,** *491* (*1944*); G. W. Thorn *et al.*, *Ibid.* **24,** *802* (*1945*)). Little is known, however, of the nutritive value of albumin in man. It was found to be deficient in tryptophan and isoleucine for the growth of young rats but maintained nitrogen balance in adult dogs when fed at a fairly low level (D. M. Hegsted, A. L. Hay, and F. J. Stare, *Ibid.* **24,** *657* (*1945*)). R. D. Eckhardt *et al.* (*Ibid.* **27,** *119* (*1948*)) administered albumin orally and intravenously to 5 normal males as a sole source of nitrogen. Comparisons were made between the albumin and lactalbumin orally and a partial protein hydrolysate "Amigen" intravenously. Daily nitrogen balance was followed as well as frequent determinations of blood hematocrit, plasma volume, and plasma protein concentrations. The basal diet furnished from 3000 to 3300 calories and only from 0.1 to 0.3 g. of nitrogen daily.

Fifty g. of albumin by mouth daily were found to maintain nitrogen balance and weight in one individual over a period of ten days, while in 2 other subjects 37.5 g. of albumin alone, or when supplemented with tryptophan and isoleucine, led to persistent negative nitrogen balance and loss of weight. A negative balance was also observed in both subjects throughout an eight day period in which 37.5 g. of lactalbumin were given by mouth. The data suggest that, if given in quantities of 50 g. daily and with adequate calories, human albumin is able to maintain nitrogen balance and weight in normal adults as the sole source of protein administered.

During the periods of oral albumin administration the nitrogen excretion equaled or exceeded the intake and was considerably greater than minimal. The plasma albumin concentration did not change significantly. Albumin administered intravenously for four, eight, and sixteen days in 3 subjects (37.5 or 75 g. daily) gave different results. Nitrogen excretion was low, at almost minimal levels, and the plasma albumin concentration rose progressively reaching a maximum of 6.1 g. per 100 ml. in one patient. In 2 individuals, oral supplements of tryptophan and isoleucine during part of the period of intravenous albumin administration did not alter the positive nitrogen balance observed. As expected, the plasma volume rose during intravenous albumin administration, as did the total circulating albumin. Following cessation of albumin administration the total circulating albumin gradually dropped and could be plotted on a four or six day 50 per cent disapperance curve. Return to the control values required approximately three weeks.

In contrast to the periods of intravenous administration of albumin were the results of periods of casein hydrolysate administration in equivalent amounts, during which nitrogen balance was usually close to

equilibrium. The authors found that about 45 per cent of the conjugated amino acids (polypeptides) of "Amigen" administered intravenously were excreted in the urine. This confirms the work of H. N. Christensen and co-workers (*Nutrition Reviews* **6,** *53 (1948)*). In a short period of oral albumin following a period when it was given intravenously the nitrogen excretion rose rapidly until on the third day approximate nitrogen equilibrium ensued.

From their results and from those of others the authors conclude that albumin administered intravenously first diffuses into the lymphatics producing a rise in both plasma and lymph albumin concentration after repeated injections. Following cessation of albumin administration the reverse occurs and the disappearance of albumin from the plasma may be used as a measure of the disappearance of albumin from both compartments. The authors also believe that the albumin which disappears does so by breaking down to its component amino acids which are either converted into tissue protein or deaminized and excreted, depending upon the body needs. The authors believe that in this way parenterally administered albumin acts as a presumably good body protein nutrient but is available for nutritional purposes much more slowly than protein by mouth or hydrolyzed protein intravenously. However, this study does not answer adequately what one really wishes to know about the nutritive value of albumin in man. In the original study on the nutritive value of albumin in rats and dogs (Hegsted *et al., loc. cit.*), the tremendous difference in amount required by the two types of animals was emphasized. It is still unknown whether there is a species difference or whether the difference in requirement was simply because the rats were in the growing stage while the dogs were adults. In any event, the latter difference is undoubtedly an important factor since it is known that in the same species protein requirements for growth are much higher than those for maintenance. The studies by Eckhardt *et al.* are similar to those done with dogs., i.e., maintenance in normal adults, and it is doubtful whether these results can be carried over to those cases who have suffered serious protein depletion due to various causes where albumin supplementation is really important. In such cases tissue must be formed, a situation which in all likelihood is comparable to growth. Whether albumin in convenient amounts will be sufficient in such circumstances, or whether isoleucine and tryptophan supplementation might then be advantageous is still unknown.

PANTOTHENIC ACID ANTAGONISTS

Antimetabolites related to pantothenic acid have been reported in great number since pantoyltaurine was found to be an effective *in vitro* antagonist against this vitamin. The total number prepared probably exceeds that of any group except those related to sulfanilamide.

Recent additions to the list of pantothenic acid analogues have come about in part as a result of the report by W. Trager (*J. Exp. Med.* **77,** *411 (1943)*) that Plasmodium lophurae required this vitamin for growth. J. F. Mead and co-workers (*J. Biol. Chem* **163,** *465 (1946)*) prepared pantoyltaurine and a number of its derivatives in which the functional atoms of the sulfonic acid group were replaced by ammonia, sodium, and several amines. Of these, d-pantoyltauryl-amide was found to be active *in vivo* in the suppression of the malarial parasites, although the degree of efficacy was not stated.

R. Winterbottom and co-workers (*J. Am. Chem. Soc.* **69,** *1393 (1947)*), working along the same lines prepared twenty-five deriva-

tives of pantoyltaurine and fifty-one derivatives of taurine in an effort to obtain compounds with better absorption-excretion characteristics than pantoyltaurine itself. Reports of the testing of taurine derivatives were not given, and these were presumably without activity. However, pantoyltaurine and its amides showed high antibacterial and antiplasmodial activity *in vitro*; against Streptococcus hemolyticus many of the derivatives were reported to be much superior to the parent drug. The derivatives were similar to pantoyltaurine in the superiority of the d-isomer over the l-, the latter being without activity wherever tested. Pantothenic acid completely overcame the effect of these drugs, indicating that they functioned to inhibit the utilization of the vitamin.

When administered in a single oral dose, many of the analogues gave therapeutically effective blood levels. The two most desirable compounds for the treatment of S. hemolyticus in mice were the 4-chlorophenyl and 3,5-dibromophenyl amides, which possessed antibacterial indices of 25 and 12, respectively (molar ratios of inhibitor to metabolite necessary to inhibit growth). Against trophozoite-induced P. gallinaceum infections in chicks, fifteen of the analogues showed antimalarial activity. The most potent was the 4-chlorophenyl derivative above, which was four to sixteen times as effective as quinine. All were reported to be markedly superior to pantoyltaurine, which showed no activity when injected in large intraperitoneal doses. The authors were uncertain whether the differences in activity were caused by variation in inherent antimalarial activity or in pharmacologic characteristics.

Growth reversals involving pantothenic acid have been used by workers in two laboratories to study the attachment of the vitamin within the cell and its mode of action in metabolism. V. H. Cheldelin and C. A. Schink (*J. Am. Chem. Soc.* **69,** *2625 (1947)*) prepared three analogues of pantoic acid and their beta-alanides, and compared their effect upon the growth of microorganisms with the effect produced by pantoyltaurine and similar compounds in which the beta-alanine portion of the molecule had been altered. Removal of either of the hydroxy groups in pantoic acid produced good inhibitors for Acetobacter suboxydans; the compound without the alpha-hydroxy group reduced growth 50 per cent at a concentration ten times that of the metabolite. These analogues were less effective against yeast and not at all against L. arabinosus. When the beta-alanides were tested, however, they exhibited varying degrees of vitamin activity, being up to 90 per cent as active as beta-alanine for yeast. It was considered likely that yeast was capable of hydrolyzing the analogues to produce beta-alanine, which serves as a source of the vitamin for this organism.

The contrasting behavior of the pantoic acid analogues and their beta-alanides was in turn different from the effects produced by analogues in which the beta-alanine moiety had been altered. In general, compounds of the latter type were excellent inhibitors, particularly in organisms requiring the preformed vitamin. The authors interpreted these findings as indicating that pantothenic acid is normally attached to cellular enzymes through one or both of its hydroxy groups, with alteration of these groups greatly reducing the biologic activity. Compounds such as pantoyltaurine were thought to be capable of attachment but incapable of subsequent biologic function due to alteration of essential groups in the beta-alanine structure.

J. M. Ravel and W. Shive (*J. Biol. Chem.* **166,** *407 (1946)*) reported that in Escherichia coli cysteic acid seemed able to prevent the decarboxylation of aspartic acid to beta-alanine, thereby resulting in lowered pantothenic acid synthesis and reduced growth. In a later paper W. Shive, W. W. Ackermann, J. M. Ravel, and J. E. Sutherland (*J. Am. Chem. Soc.* **69,** *2567 (1947)*) reported

that citric, cis-aconitic, or alpha-ketoglutaric acid were able to raise the antibacterial index of cysteic to aspartic acid approximately tenfold. The authors concluded that these components of the Krebs cycle allowed growth to proceed at a lower rate of pantothenic acid synthesis, and that a normal role of pantothenate was to mediate the formation of cis-aconitate. In support of this view they cited the reversing effect of both pantothenic and alpha-ketoglutaric acids upon a pantothenic acid antagonist for E. coli, as well as the sparing action of alpha-ketoglutaric acid upon the pantothenate requirement of Proteus morganii.

F. Lipmann and N. O. Kaplan (*Nutrition Reviews* **5,** *331 (1947)*) have presented evidence which suggests that pantothenic acid functions in the metabolism of acetate. This concept is supported by an experiment by Shive and co-workers using L. arabinosus, which is believed to utilize acetate in the formation of fatty acids and sterols. Both oleic acid and sodium glycocholate were found to reduce the effectiveness of pantoyl-n-butylamine as an inhibitor of pantothenic acid for this organism, and it was suggested that the inhibitor may have prevented the conversion of acetate to compounds of these types. Although experiments such as these do not themselves furnish complete evidence of the biochemical role of pantothenic acid, they are valuable in providing clues which in this case tend to support the interpretations formed from more direct studies of enzyme systems, and they illustrate the possible applications of inhibitors to studies of other metabolites.

DENTAL CARIES EXPERIENCE IN MALNOURISHED CHILDREN

A low lifetime dental caries experience in two groups of malnourished individuals has been reported by A. W. Mann, S. Dreizen, T. D. Spies, and F. M. Hunt of Birmingham, Alabama (*Nutrition Reviews* **5,** *178 (1947)*). These studies were difficult to evaluate in terms of dental caries resistance or susceptibility since all data presented were based on lifetime dental caries experience and not on the increment of new carious lesions during a period of known nutritional deficiency. The further disadvantage of not knowing the dietary and medical history of these individuals during the periods of tooth development could not be overcome.

As a continuation of this problem, S. Dreizen, A. W. Mann, T. D. Spies, and T. A. Skinner (*Am. J. Dis. Child.* **74,** *265 (1947)*) described a clinical study where the dental caries increment was determined for two groups of malnourished children and for a group of well nourished children of roughly comparable age. The number of decayed, missing, and filled tooth surfaces at the beginning of the experiment and the increment of new carious lesions during the observation year were determined for 72 children with clinical evidence of nutritional deficiency diseases and for 25 well nourished children. Each of the malnourished children had been examined periodically in the nutrition clinic at Birmingham, Alabama for several years prior to the time this investigation was begun. The diets of the mothers of these malnourished children usually had been inadequate during pregnancy and lactation. Few of the children received orange juice or vitamin D supplements during childhood. As the children grew older, they usually had poor appetites, ate irregularly, and many preferred high carbohydrate foods. These 72 children were divided into two groups: group 1 was composed of 47 children who were malnourished throughout the observation year, while group 2 was composed of 25 malnourished children who received a quart of milk daily for six days a week during an eighteen month period which included the observation year. The control group consisted of 25

well nourished children who were fed an adequate diet and who were free from evidence or past history of nutritional deficiency diseases. The mothers of these children had been under the care of physicians during the prenatal period in contrast to the lack of professional advice for the mothers of the malnourished children during pregnancy. The average age of the children in the three groups was 11.2, 10.8, and 10.4 years, respectively. Of the 47 children in group 1, "44 had clinical evidence of riboflavin deficiency; 2, of nicotinic acid deficiency; 4, of thiamine deficiency; 4, of ascorbic acid deficiency; and 9, of vitamin A deficiency." Of the 25 in group 2, "20 of the children had signs and symptoms of riboflavin deficiency; 2, of thiamine deficiency; 12, of ascorbic acid deficiency; and 5, of vitamin A deficiency. Thirty of the children in groups 1 and 2 showed evidence of multiple deficiency disease, which is common among malnourished persons. No evidence of rickets was seen in any of these children." The carbohydrate intake per child per day was 248, 253, and 296 g., respectively, for groups 1 to 3. These values constituted 54, 55, and 49 per cent, respectively, of the total caloric intake. The average refined carbohydrate intake per day per child was 52, 48, and 67 g., respectively. These values constituted 12, 11, and 11 per cent, respectively, of the total caloric intake. The average value for the number of decayed, missing, and filled tooth surfaces for the children in groups 1, 2, and 3, respectively, were 4.52, 2.80, and 7.34 at the beginning of the experiment. The average increases in the number of carious tooth surfaces per child during the observation year were 0.95, 0.96, and 2.38, respectively. In other words, the incidence of new carious lesions in the group of malnourished children and in the group of malnourished children which received a quart of milk six times a week throughout the observation period was only 44 per cent of that observed in the well nourished group. Examination of the data for deciduous teeth indicated no difference in the susceptibility to carious lesions.

The data presented in the above study appear to answer some of the questions raised about the report of Mann *et al.* (*loc. cit.*), especially with regard to the actual number of new carious lesions developed during an observation period in these deficiency states. Additional data are presented on the comparative total and refined carbohydrate intakes of the three groups of children which were not presented in previous reports. The groups of malnourished children had somewhat less total and refined carbohydrate intakes per day on a gram basis and about the same amounts on a percentage basis as the well nourished children. An unexplained point in the data presented was the fact that the two groups of malnourished children had almost identical carbohydrate and caloric intakes despite the fact that the second group received a supplement of 1 quart of milk per day which would contribute about 44 g. of natural carbohydrate and 624 calories.

In addition, no comment was made on how this supplement affected the nutritional status of the children receiving it. Both of these items would seem to require some explanation to make a reliable evaluation of the dental caries increments during the experimental period. On the basis of present knowledge, it is not possible to explain the lower dental caries increments among the malnourished children in this study, especially since we know so little of the various foodstuffs which made up the bulk of the diets and which may have been of greater importance in the development and maintenance of intact teeth than the deficiencies recorded in the malnourished children. At present, nutrition investigators think of an adequate diet for children as one which permits normal growth, normal hemoglobin levels, and physical well-being. Whether the day will ever come when we can prescribe a diet which fulfills these objectives and at the same time prevents the development of carious lesions completely is not known (*Nutrition Reviews* **5**, *67* (*1947*)).

The data of Dreizen *et al.* suggest strongly that reductions in susceptibility to tooth decay did occur in the groups of malnourished children under investigation. The mechanism through which these reductions have occurred is of importance for further investigation. Were the differences between the dental caries increment for well nourished and malnourished children due to oral environmental differences, systemic differences attributable to the diet, or to different caries susceptibilities of the teeth resulting from variations in the diet during tooth development?

In contrast to the apparent reduced dental caries susceptibility described above, endemic pellagra is unquestionably characterized by the development of an extensive gingivitis and stomatitis which begins at the interdental papillae and progresses rapidly and extensively until the gingivae become tender, painful, and often ulcerating (*Nutrition Reviews* **4,** *41* (*1946*)). The ultimate result of the invasion of such lesions by microorganisms is the breakdown of the supporting structures of the teeth to form periodontal lesions of various types. The prevalence of periodontal disease in malnourished patients has been recorded by A. W. Mann, J. M. Mann, and T. D. Spies (*Ibid.* **4,** *139* (*1946*)) who stated that periodontal disease had been the chief cause of tooth extraction in 105 of their 160 edentulous patients who had had longstanding records of multiple deficiency disease. This possible inverse relation between the processes of dental caries and of periodontal disease suggests that the breakdown products of tissue proteins during the formation of periodontal lesions might be one pathway through which the susceptibility of dental caries might be altered. Interesting studies *in vitro* and with experimental animals could be made to test this possibility.

MIXED DEFICIENCY DISEASES IN INDIA

An enlightening clinical description of deficiency diseases encountered in India has been presented by R. Passmore (*Tr. Roy. Soc. Trop. Med. Hyg.* **41,** *189* (*1947*)) who is Director of the Nutrition Research Laboratories, Coonoor, South India. Dr. Passmore notes that the symptom complexes commonly described under the headings of beriberi, pellagra, scurvy, rickets, and osteomalacia are rare except for small outbreaks confined largely to certain population groups or limited geographic areas. In contrast to this, other evidences of deficiency states are widespread. One of the most important, and frequently overlooked of these, is loss of weight in an adult or failure to gain weight in a child. In discussing the importance and simplicity of this observation, Passmore states ". . . recent medical literature contains many descriptions of complex chemical and physiological tests, involving elaborate apparatus for assessing nutrition and has emphasized the difficulties and errors that may be met. As a result many doctors, ignorant of the principles involved in such tests and without the means of carrying them out, have lost confidence in themselves and fail to use the simple observational methods."

The numerous skin manifestations of defective nutrition are discussed, including "crazy-pavement skin," phrynoderma or toad-skin, and degeneration of the junction of the skin and mucous membranes affecting the lips, nostrils, eyelids, external auditory meatus, and genital and anal regions. This latter defect is termed "orogenital syndrome." It is noted that these various skin lesions are not necessarily specific for single vitamin deficiencies, but that some may respond to administration of one factor while others respond to therapy with another dietary essential. Similarly, it is noted that nutritional glossitis and diarrhea may respond at times to administration of niacin, at other times to riboflavin, but

many cases require other unidentified members of the vitamin B-complex.

This worker observes that the keratitis, photophobia, circumcorneal injection, and corneal vascularization which are often attributed to riboflavin deficiency, are not commonly associated with severe malnutrition as observed in general hospitals in India or Bengal. Xerophthalmia and nutritional night blindness occur and respond to administration of vitamin A.

It is stated that "vitamin B_1 (thiamine) has been given both by mouth and subcutaneously to many patients with peripheral neuritis and an associated malnutrition. No evidence has been obtained that the vitamin has any effect on the rate of recovery from the neuritis." This indicates that factors other than thiamine deficiency are of importance in the neuritis commonly encountered in India.

Passmore concludes that the reports of deficiency diseases in India and elsewhere have generally been misleading because they have usually been limited to description of lesions in one system of the body or to attempts to describe the picture of a deficiency resulting from the lack of a single dietary factor. In contrast to these pictures, the diseases as ordinarily encountered vary greatly in detail and every degree of severity from a minor sign which may go unnoticed by the patient to severe irrecoverable diseases may be met. The syndromes may be composed of numerous associated deficiencies, which is what one would expect, and which is why therapy with mixtures of nutrients or food is generally desirable.

GOITER ON AN IODINE FREE DIET

The provocative article by I. Greenwald dealing with the pathogenesis of endemic goiter was discussed in an earlier issue (*Nutrition Reviews* **6**, *40, 159 (1948)*). In this paper the conclusion was reached that endemic goiter is not due to a lack of iodine. In view of this criticism of the classic theory of the pathogenesis of endemic goiter, a report by J. F. McClendon and W. C. Foster (*J. Clin. Endocrinology* **7**, *714 (1947)*) is of interest. These workers set out to observe the effect of an iodine free diet under conditions in which exposure to a hypothetical goiter-producing agent might be excluded. The experiment was conducted in Philadelphia, a region where goiter is not endemic. Food for the animals was grown by hydroponics in a disinfected greenhouse. Persons or animals with goiter were not permitted inside the greenhouse where the food was grown. Precautions were taken against the survival of insects and the water used was rain caught on a glass roof and filtered in such a way that organisms might be excluded. Seeds of corn, soybeans, and sunflowers were sprouted in broken quartz that had been heated to 150°C. in sulfuric acid, a process that would kill any organisms. The seedlings were transplanted through holes in the aluminum covers of the tanks and the entering air was passed through copper screen. Even the air to the roots of the seedlings was filtered through 2 inches of packed asbestos followed by carbon filter tubes.

A diet so prepared was fed to rats of a Wistar colony that had been isolated for six years from goitrous animals or man. At the end of seventy-three days the rats were anesthetized, the thyroids dissected out, placed on small round, previously weighed cover glasses and weighed on a chainomatic balance. The rats on this diet and redistilled water had thyroid glands weighing 41, 42, and 39 mg. per 100 g. of body weight whereas the rats on the same diet supplemented with water containing iodine had normal thyroids weighing 10 mg. per 100 g. of body weight.

These differences are admittedly striking, even though only 6 rats were used in the study. The conclusion is drawn that goiter is produced primarily by a lack of iodine.

NOTES

Letter to the Editor

Dear Sir:

In a recent issue (*Nutrition Reviews* **6,** *40 (1948)*) you closed a review of a paper of mine with the words "his contention that iodine lack has nothing to do with goiter is entirely untenable." I made no such contention. I did conclude that "endemic goiter is not due to a lack of iodine."

You express surprise "to find in such an extensive modern review no mention of the long-known and widely accepted role as goitrogenic agents of some foods and dietary factors. . ." I made no attempt to review all the hypotheses concerning the cause of goiter. I was concerned with only one, which I believe rests upon no valid evidence whatever and is contradicted by a large number of observations.

You follow Kimball in denying the validity of my corollaries. You state that a normal metabolic rate "would be expected if the hypertrophied gland suffices to meet the demands for thyroid hormone." This is supporting one hypothesis by producing another.

You also object that many of the data in my table were obtained from analyses of normal thyroids. Why are they not pertinent? If a marked lack of iodine leads to an unmistakable goiter, should not a lesser deficiency lead to a somewhat enlarged gland and should there not be a negative correlation between the size of the gland and its iodine content? Moreover, what is a "normal" thyroid?

You criticize my use of the data showing that the iodine contents of different varieties of the same vegetable grown upon the same soil and at the same time, differ as much, or more, as has ever been claimed to exist between the products of goitrous and nongoitrous regions, saying "he is considering single instances and not average of several samples grown over a region." Most of the analyses purporting to show differences between goitrous and nongoitrous regions are similarly concerned with single samples.

As regards the prophylactic use of iodides, you state "Kimball, however, who directed the surveys from which Greenwald quotes, states that the inferences which he has drawn from them are 'terribly wrong'." Kimball's judgment as to the validity of my inferences are not, *ipse facto*, more correct than mine. Moreover, he directed only some of the surveys from which I quoted.

You ignore completely my argument that the food consumed by the population of our large cities varies only slightly from city to city. Finally, you neglect to mention that the paper under discussion was preceded by another, which embodied the results of a review of the history of goiter in the Americas, in New Zealand, and in England (*Bull. Hist. Med.* **17,** *229 (1945)*) from which I concluded that goiter did not exist in the Americas or in New Zealand before the coming of white men, nor in England before 1700, and that its history was that of an epidemic disease, of varying severity and incidence.

ISIDOR GREENWALD
New York University College of Medicine

Community Nutrition

The Bureau of Agricultural Economics, U. S. Department of Agriculture, working jointly with the Nutrition Programs Office, has sponsored an intensive study of "Homemakers' Acceptance of Nutrition Information in an Urban Community" in the area of Richmond, Virginia.

The reports from this study will be of interest to nearly everyone concerned with lay education in regard to nutrition and health. For example, among major findings, it was apparent that:

(1) "Half the homemakers have little or no information on nutrition. A very small

proportion (4 per cent) of these homemakers were rated as having adequate information on nutrition. The remaining 46 per cent were rated as having some information on nutrition."

(2) "About one-half the women included foods from all the seven basic food groups in their daily menu."

(3) "Homemakers showed more interest in information about food and its preparation than in nutrition."

(4) "Most homemakers said they got most of their ideas on food or nutrition from newspapers and magazines."

(5) "Although one-half the women said they had heard of classes or meetings on nutrition, only one-sixth said they had ever attended."

(6) "Mothers of almost half the children attending school attributed some nutrition information to their children's experience in school."

Motivating factors stated to be of greatest effectiveness were:

(1) "... the foods they should eat are better for the health and growth of their children."

(2) "... if they and their families eat the foods they should, they will have more energy and live longer."

(3) "... point out to them the importance to health of the foods they should eat."

It was astounding to find that practically no headway had been made in this area toward improvement in status of nutritional education between the periods 1943 and 1947. As one would expect, educational progress had been greatest in the middle and upper thirds of the groups classified on an economic basis.

(Mimeographed bulletin; 71 pages; may be obtained from the United States Department of Agriculture, Bureau of Agricultural Economics, Washington, D. C.; dated February, 1948.)

Biosynthesis of Methionine

The connection between genes and the chemical reactions which an organism can perform has already received attention in this journal (*Nutrition Reviews* **4,** *326 (1946)*; **5,** *270 (1947)*).

Further work with the mold Neurospora has been performed by N. H. Horowitz (*J. Biol Chem.* **171,** *255 (1947)*) who has studied methionine synthesis in the organism. The work of V. du Vigneaud, G. B. Brown, and J. P. Chandler (*Ibid.* **143,** *59 (1942)*) showed the path of methionine synthesis in the rat, and the possibility was considered that similar reactions might occur and be under gene control in the mold.

A preliminary survey was therefore made of mutant forms of Neurospora and four were found which were unable to grow on the usual sulfur free medium with added methionine. Of these four, strain *me-1* could not utilize homocystine while the other three could. The present study indicated that the conversion of cystine to methionine takes place in Neurospora in a manner different from that previously described in the mammal. There is evidence that cystathionine is an intermediate since this was actually formed and precipitated from the growth medium when strain *me-2* was grown. This may therefore be presumed to be formed in the conversion of cysteine to methionine in Neurospora of the wild-type (unirradiated genes), although direct proof is lacking.

Recent Nutrition Book

Appraisal of Human Dietaries by Animal Experiment. A conference under the Auspices of the Williams-Waterman Fund for the Combat of Dietary Diseases. March 13, 1947, Hotel Roosevelt, New York. Edited by Robert R. Williams, Chairman of the Fund and Conference. Published by the Williams-Waterman Fund Research Corporation, 405 Lexington Avenue, New York 17, New York. Pp. 130.

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THE NUTRITION FOUNDATION

The Nutrition Foundation was organized by food and related manufacturers in December, 1941, as a sincere expression of their interest in scientific progress and human health. The basic purposes of the Foundation are: (1) the development of a comprehensive program of fundamental research, providing basic information in the science of nutrition; and (2) the support of educational measures that will assist in making the science of nutrition effective in the lives of present and future generations.

The publication of NUTRITION REVIEWS has been undertaken to enable professionally trained people to keep abreast of current progress, and to have available an unbiased, authoritative review of the current research literature in the science of nutrition.

The Editorial Staff has been carefully selected so that the publication will merit in reasonable degree, the confidence of the medical profession and others who work directly with the public in the field of nutrition. The service provided by the publication is distinct from that of an abstract journal, a review for laymen, or an extensive scientific review. Since the reviews as originally prepared by a member of the Editorial Staff are frequently modified by special referees and may be further modified by the Editorial Office in preparing final copy for the press, the Editorial Committee feels that it is preferable not to affix the names of Editorial Staff members to individual reviews. There is thus a high degree of group responsibility on the part of the Editor and Assistant Editors.

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